Signa Vitae

REVIEW

Therapeutic agents for ARDS

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Abstract

In spite of supportive care of patients with acute respiratory distress syndrome (ARDS), morbidity and mortality of these patients are considerable and effective therapies centred in ARDS pathophysiology are needed. Substantial progress in pharmacological therapies has been noticed, however, several studies have not been successfully translated to the clinics. Nonetheless, many preclinical and clinical studies are ongoing. In this review, pharmacological therapies underlying ARDS pathophysiology are summarized: therapies targeting the alveolocapillary membrane, mucolytics, bronchodilators, immunomodulators, anticoagulants and fibrinolytics, aspirin, and other treatments are discussed, including both, studies with beneficial and controversial results, and ongoing trials. In addition, a section concerning preclinical studies is included. An enlarged understanding of ARDS pathophysiology and its fundamental pathways and mechanism, together with the identification of ARDS subsets of patients and phenotypes will maximise patient response to a specific treatment.

Keywords

Acute respiratory distress syndrome (ARDS); Acute lung injury; COVID-19; Sepsis

1. Introduction

Acute respiratory distress syndrome (ARDS) is an acute hypoxemic respiratory failure in critically ill patients of all ages [1]. This syndrome may originate from multiple insults that affect directly the lung (pneumonia or aspiration of gastric contents, among others), or systemic insults that will develop ARDS as a consequence of the primary disease (sepsis or trauma, among others) [1]. Recent clinical ARDS categories include patients with Coronavirus disease 2019 (COVID-19) caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) [2]. ARDS is heterogenic based on its etiology, illness severity, duration, and individual patient characteristic, determining the course of the disease. Morbidity and mortality of ARDS remain high [3, 4], about 35–40%. Most surviving patients experience persistent and prolonged physical, mental and quality-of-life impairment, requiring specific medical attention after recovery of ARDS [5].

The pathophysiology of ARDS is characterized by the breakdown of the alveolar-capillary barrier, which leads to proteinaceous edema and neutrophils infiltration into the alveolar compartment, with pulmonary activated coagulation and inflammation, and decreased fibrinolysis [1, 6, 7]. Nowadays there is no single biomarker able to identify ARDS nor its underlying biology.

Currently, the management of ARDS patients is mainly supportive and preventive, and specific effective pharmacological therapy is not available yet. Despite years of research and knowledge, several preclinical and clinical studies have not been successfully translated. However, science is increasingly advancing day by day, and many treatments focused on ARDS pathophysiology are underway, and many others have emerged during the actual COVID-19 pandemic. Progressive understanding of the pathways and mechanisms involved in this disease, together with the identification of subsets of patients underlying ARDS might improve treatment response.

This narrative review is focused on the pharmacological therapies that have been proposed to treat adult ARDS, highlighting their beneficial and controversial effects, especially on those therapies that are ongoing but without excluding those that did not work. To better understand the mechanisms of the different therapies for adult ARDS, in some sections, studies on neonate/pediatric ARDS (soluble guanylate cyclase surfactant, budesonide) or studies for sepsis (Bevacizumab, Levosimendan Hydrocortisone, Vitamin C, Sivelestat Sodium, anti-TF antibody-836 (ALT-836), Antithrombin, thrombomodulin alfa-123 (ART-123), Drotrecogin alfa) have been introduced. Clinical studies have been found in Home-Clinical Trials. gov or PubMed (nih.gov).

The article is divided into therapies targeting the alveolocapillary membrane, mucolytics, bronchodilators, immunomodulators, anticoagulants and fibrinolytics, aspirin, and other treatments, including data of relevant preclinical and clinical studies and highlighting those that are ongoing (Fig. 1, Table 1). The different therapies are classified according to their main actions on target key processes and pathways of ARDS complex pathophysiology, but this does not exclude that one therapy do exert its effects through different systems. Also, there is a section for preclinical treatments which have not been

Mucolytics
Deoxyribonuclease-1

Bronchodilators

Alveolocapillary membrane
ACE2
Alveolar epithelium
Surfactant
AP301
Keratinocyte Growth Factor
Alveolar endothelium
Nitric Oxide Synthase
Prostacyclin
Anti-Vascular Endothelial Growth Factor
Levosimendan

Immunomodulators
Neuromuscular blockers
Steroids
Statins
Carbon monoxide
Mesenchymal Stromal Cells
Regulatory T-cells
Vitamin C
Ulinastatin
Inhibitors
p38
Tumor Necrosis Factor Receptor 1
Interleukin-6
Interferons
Imatinib
NLRP-3 Inhibitors
Granulocyte-macrophage colony-stimulating factor
Neutrophil –proteases inhibitors

Alveolar Compartment

Inflammation

Alveolar Macrophage

Coagulation

Fibrin deposition

Neutrophil infiltration

Alveolar Epithelium

edema

Alveolocapillary membrane Breackdown

Anticoagulants and Fibrinolytics
Tissue Factor
Antithrombin
Heparin
Thrombomodulin
Activated Protein C
Streptokinase

Fibroblast

Endothelium

Platelets

Aspirin

Preclinical therapies for ALI
Adenosine A2A receptor agonists
Proteinase-activated receptor 1
Receptor for advanced glycation end-products inhibitors
Haptoglobin
Lipoxin A4

Others
Alpha-1 antitrypin
Transient Receptor Potential Vanilloid 4 inhibitor
Matrix metalloproteinase 12 inhibitor
Sevoflurane

FIGURE 1. Acute respiratory distress syndrome and proposed preclinical and clinical specific pharmacological therapies.

tested yet in clinical trials.

2. Alveolocapillary membrane

Damage into the alveolocapillary membrane drives the loss of epithelial and endothelial barrier integrity, which leads to protein-rich edema extravasation and leukocytes infiltration into the alveolar compartment [8].

2.1 ACE2

The renin-angiotensin system (RAS) is involved in ARDS pathophysiology. Patients with ARDS present increased levels of Angiotensin II, a vasoconstrictor involved in inflammation and pulmonary edema that exerts its activities through angiotensin type I receptor [9]. Angiotensin Converting enzyme II (ACE2) hydrolyses Angiotensin II producing Angiotensin 1–7, which has been found to be protective in experimental models. In a randomized phase 2a clinical study, GSK2586881 (recombinant human ACE2) was administered as an exogenous ACE2, in order to hydrolize Angiotensin II, and proved safety but did not improve clinical outcomes in ARDS patients requiring mechanical ventilation [10].

In patients with COVID-19, SARS-CoV-2 is known to bind ACE2; both membrane-bound (mACE2) and soluble (sACE2) forms. However, only mACE2 mediates the virus entrance into the cell, but not sACE2. Angiotensin type I receptor blockers increase the levels of Angiotensin II, which stimulates ACE2 shedding; sACE2 catalyzes the conversion of Angiotensin II to Antiotensin 1–7 while also binds SARS-CoV-2 blocking its entrance to the host cells. Presently, there is an ongoing randomized phase 2 trial with oral 50 mg Losartan (an angiotensin type I receptor blocker) and 25 mg Spironolactone (a blocker of aldosterone secretion) in patients with COVID-19-ARDS (NCT04643619).

2.2 Alveolar epithelium

The alveolar epithelium has a key role in ARDS severity [11]. It is composed of alveolar type I cells (ATI cells), which cover the 95% of alveolar surface and are the major responsible of gas exchange, and alveolar type II cells (ATII cells), which are the progenitor cells of the alveolar epithelium and can proliferate and differentiate into ATII cells. ATII cells also produce surfactant. Both cell types are critical in ion transport and present immunologic functions [12, 13].

2.2.1 Surfactant

ATII cells produce and recycle pulmonary surfactant, which is composed of proteins and lipids. Surfactant maintains the alveolar surface tension and presents antimicrobial and host defense functions [14]. ATII cells injury together with the presence of proteins and enzymes in the edema induce surfactant dysfunction [15].

In pediatric patients, exogenous surfactant evidenced benefits. In the ULTRASURF randomised controlled trial, the lung ultrasound scores improve the time of surfactant administration and prove better oxygenation after early treatment with surfactant in premature newborns [16].

In a randomized controlled trial, continuously nebulized synthetic surfactant for five days in patients with sepsis-induced ARDS did not impact 30-day survival, duration

T A B L E 1. Acute respiratory distress syndrome clinical studies of pharmacological therapies.

Therapeutic agent	Route of Administration	Mechanism of action	Severity of ARDS	Trial state	Stage of testing	Reference/ Identifier
ALVEOLOCAPILLARY MEMBRANE						
RAS related signalling						
Recombinant human ACE2 (GSK2586881)	Intravenous	Cleavage of Angiotensin II to Angiotensin 1–7	ARDS patients requiring mechanical ventilation for <72 h	Phase 2	Completed	[10]
Losartan and Spironolactone	Oral	Blocking angiotensin receptor and secretion of aldosterone.	COVID-19 ARDS	Phase 2	Recruiting	NCT04643691
Alveolar epithelium						
Synthetic surfactant	Aerosolized	Replace surfactant	Sepsis-induced ARDS	Not Applicable	Completed	[17]
Recombinant surfactant protein C	Intratracheal	Replace surfactant	Various etiologies	Phase 3	Completed	[18–20]
Poractant Alfa	Through bronchoscopy	Replace surfactant	COVID-19 ARDS	Not Applicable	Completed	[21]
Poractant Alfa	Endotracheal instillation or bronchial fibroscopy	Replace surfactant	COVID-19 ARDS	Phase 2	Recruiting	NCT04502433/ NCT04384731
AP301 (Solnatide)	Inhaled	Activation alveolar epithelium Na^+ channels	ARDS patients requiring mechanical ventilation	Phase 2a	Completed	[24, 25]
AP301 (Solnatide)	Inhaled	Activation alveolar epithelium Na^+ channels	Moderate-to-severe ARDS	Phase 2b	Recruiting	NCT03567577
Recombinant hKGF (palifermin)	Intravenous	ATII cell proliferation, migration, and regeneration	Not specified	Phase 2	Completed	[29]
Alveolar endothelium						
L-citrulline	Intravenous	Substate of NOS	Sepsis-induced ARDS	Phase 2	Completed, unpublished	NCT01474863
L-citrulline	Oral, dietary supplement	Substrate of NOS	COVID-19 ARDS	Not Applicable	Completed, unpublished	NCT04404426
sGC activator (BAY 1211163)	Inhaled	Conversion of GTP into cGMP	Moderate or severe ARDS	Phase 1	Recruiting	NCT04609943
Iloprost	Inhaled	Prostacyclin analogue, vasodilatation	Not specified	Phase 3	Completed, unpublished	NCT03111212
Epoprostenol	Inhaled	Prostacyclin analogue, vasodilatation	COVID-19 ARDS	Phase 2	Completed, unpublished	NCT04452669
Iloprost	Inhaled	Prostacyclin analogue, vasodilatation	COVID-19 ARDS	Phase 2	Recruiting	NCT04445246

T A B L E 1. Continued.

Bevacizumab	Intrvenous	Anti-VEGF	Sepsis-induced ARDS	Phase 2	Withdrawn	NCT01314066
Bevacizumab	Intrvenous	Anti-VEGF	COVID-19 ARDS	Phase 2	Completed, unpublished	NCT04275414
Levosimendan	Intravenous	K^+ channel activator	Sepsis	Phase 2	Recruiting	NCT04020003
MUCOLYTICS						
N-acetylcysteine	Intravenous	Mucolytic	ARDS patients requiring mechanical ventilation	Not Applicable	Completed	[41]
N-acetylcysteine	Intravenous	Mucolytic	Moderate-to-severe COVID-19	Not Applicable	Completed	[42]
Dornase alfa	Inhaled	Cleaving extracellular DNA in NETs	COVID-19 ARDS	Phase 3	Terminated	NCT04355364
Dornase Alfa	Inhaled	Cleaving extracellular DNA in NETs	COVID-19 ARDS	Phase 2	Recruiting	NCT04402944
BRONCHODILATORS						
Salbutamol	Intravenous	Beta-adrenergic agonist	ARDS patients requiring mechanical ventilation	Not Applicable	Completed	[45]
Salbutamol	Intravenous	Beta-adrenergic agonist	ARDS patients requiring mechanical ventilation, ICU	Phase 2	Completed	[46]
Salbutamol	Intravenous	Beta-adrenergic agonist	ARDS patients requiring mechanical ventilation	Phase 2	Completed	[47]
Albuterol	Inhaled	Beta-adrenergic agonist	ARDS patients requiring mechanical ventilation	Phase 3	Completed	[48]
Dornase Alfa and albuterol	Inhaled	Beta-adrenergic agonist	ARDS patients requiring mechanical ventilation, COVID-19 ARDS	Not Applicable	Completed, unpublished	NCT04387786
NEUROMUSCULAR BLOCKERS						
Cisatracurium	Intravenous	Blocking cholinergic receptors	ICU patients	Not Applicable	Completed	[50]
Cisatracurium	Intravenous	Blocking cholinergic receptors	Moderate-to-severe ARDS	Not Applicable	Completed	[51]
IMMUNOMODULATIONS						
Steroids						
Dexamethasone	Intravenous	Anti-inflammatory and immunosupressor	Moderate-to-severe ARDS requiring mechanical ventilation	Not Applicable	Completed	[60]
Dexamethasone	Intravenous or oral	Anti-inflammatory and immunosupressor	COVID-19 ARDS	Not Applicable	Completed	[61]
Dexamethasone	Intravenous	Anti-inflammatory and immunosupressor	COVID-19 ARDS	Phase 4	Recruiting	NCT04663555
Dexamethasone and Methylprednisolone	Intravenous	Anti-inflammatory and immunosupressor	COVID-19 ARDS	Phase 3	Recruiting	NCT04499313

T A B L E 1. Continued.

Hydrocortisone	Intravenous	Anti-inflammatory and immunosupressor	Sepsis-induced ARDS	Not Applicable	Completed	[62]
Methylprednisolone	Intravenous	Anti-inflammatory and immunosupressor	Severe	Not Applicable	Completed	[63]
Methylprednisolone	Intravenous	Anti-inflammatory and immunosupressor	Severe ARDS	Not specified	Completed	[64]
Methylprednisolone	Intrapleural	Anti-inflammatory and immunosupressor	ARDS and multi-organ dysfunction syndrome	Phase 2	Completed, unpublished	NCT01423864
Canrenone	Intravenous	Diuretic	COVID-19 moderate-to-severe ARDS	Phase 2	Not yet recruiting	NCT04977960
Budesonide	Inhaled	Anti-inflammatory and immunosupressor	Not specified	Phase 2	Completed	[65]
Budesonide	Inhaled	Anti-inflammatory and immunosupressor	ARDS patients requiring mechanical ventilation	Not Applicable	Completed	[66]
Budesonide	Inhaled	Anti-inflammatory and immunosupressor	Paediatric	Phase 2	Terminated	NCT04064684
Budesonide	Intratracheal	Anti-inflammatory and immunosupressor	Neonatal severe ARDS with mechanical ventilation	Not Applicable	Completed	[67]
Budesonide and surfactant	Intratracheal	Anti-inflammatory and immunosupressor	Preterm infants	Not Applicable	Completed	[68]
Statins						
Simvastatin	Oral	HMG-CoA reductase inhibitor, immunomodulatory	ARDS diagnosed <48 h	Phase 2b	Completed	[70]
Rosuvastatin	Oral	HMG-CoA reductase inhibitor, immunomodulatory	ARDS and suspected infection	Not Applicable	Completed	[71]
Carbon monoxide						
CO	inhaled	Down-regulation NLRP3, anti-apoptotic, anti-inflammatory	Intubated patients	Phase 2	Recruiting	NCT03799874
Mesenchymal Stromal Cells						
MultiStem	Intravenous	Immunomodulatory and reparative effects	Moderate-to-severe ARDS patients requiring mechanical ventilation	Phase 1/2	Completed	[77]
hMSCs	Intravenous	Immunomodulatory and reparative effects	Moderate-to-severe ARDS patients requiring mechanical ventilation	Phase 2a	Completed	[78]

T A B L E 1. Continued.

hMSCs	Intravenous	Immunomodulatory and reparative effects	Moderate-to-severe ARDS patients requiring mechanical ventilation	Phase 2b	Recruiting	NCT03818854
CD362 enriched MSCs	Intravenous	Immunomodulatory and reparative effects	ARDS patients requiring mechanical ventilation, COVID-19	Phase 1/2	Active	NCT03042143
ACE2⁻MSCs	Intravenous	Immunomodulatory and reparative effects	COVID-19 ARDS	Not Applicable	Completed	[79]
Regulatory T-cells						
T-regulatory cells	Intravenous	Promoting homeostasis	COVID-19 ARDS	Phase 1/2	Recruiting	NCT05027815/ NCT04468971
Treg/Th2 hybrid cells	Intravenous	Decreasing Th1 response	COVID-19 ARDS	Phase 1/2	Terminated	NCT04482699
Vitamin C						
Vitamin C	Intravenous	Antioxidant	Sepsis-induced ARDS	Phase 2	Completed	[82]
Vitamin C	Oral	Antioxidant	COVID-19 ARDS	Not Applicable	Completed	NCT04570254
Vitamin C	Intravenous	Antioxidant	COVID-19 ARDS	Not Applicable	Completed	NCT04710329
Vitamin C	Intravenous	Antioxidant	Sepsis-induced ARDS	Phase 3	Not yet recruiting	NCT04404387
Ulinastatin						
Ulinastatin	Intravenous	Urinary trypsin inhibitor	ARDS patients requiring mechanical ventilation	Not Applicable	Completed	[83]
Inhibitors						
Dilmapimod	Intravenous	P39MAPK inhibitor	Patients at risk	Phase 2	Completed	[84]
Anti-TNRF1	Inhaled	Antagonizes TNF-α	LPS-induced experimentally	Phase 1	Completed	[88]
Tocilizumab	Intravenous	Blocking IL-6	COVID-19 ARDS	Phase 2/3	Completed	NCT04445272
Tocilizumab	Intravenous	Blocking IL-6	COVID-19 ARDS	Phase 3 /Not applicable	Recruiting	NCT04412772/ NCT05082714
Imatinib	Oral	Tyrosine kinase inhibitor	LPS-induced experimentally	Phase 1	Completed, unpublished	NCT03328117
Pirfenidone	Oral	Inhibition NLRP3	COVID-19 ARDS	Phase 3	Unknown	NCT04282902
Pirfenidone	Nasogastric	Inhibition NLRP3	COVID-19 ARDS, severe	Not Applicable	Recruiting	NCT04653831
Vadadustat	Oral	Increasing EPO production	COVID-19 ARDS	Phase 2	Recruiting	NCT04478071
Otilimab	Intravenous	Anti-GM-CSF	COVID-19 ARDS	Phase 2	Completed	NCT04376684
Lenzilumab	Intravenous	Anti-GM-CSF	COVID-19 ARDS	Phase 3	Completed, unpublished	NCT04351152
TJ0023234	Intravenous	Anti-GM-CSF	COVID-19 ARDS	Phase 2/3	Recruiting	NCT04341116
Sargramostim	Intravenous	Anti-GM-CSF	COVID-19 ARDS	Phase 2	Recruiting	NCT04400929
Mavrilimumab	Intravenous	Anti-GM-CSF	COVID-19 ARDS	Not Applicable	Completed	[107]
Sivelestat sodium	Intravenous	Neutrophil-proteases inhibitors	ARDS patients requiring mechanical ventilation, ICU	Not Applicable	Completed	[116]

T A B L E 1. Continued.

Sivelestat sodium	Intravenous	Neutrophil-proteases inhibitors	not specified	Not Applicable	Completed	[117]
Sivelestat sodium	Intravenous	Neutrophil-proteases inhibitors	Sepsis-induced ARDS	Phase 3	Recruiting	NCT04973670
Sivelestat sodium	Intravenous	Neutrophil-proteases inhibitors	not specified	Phase 4	Not yet recruiting	NCT04909697
Others						
SNG001 (Interferon β-1α)	Inhaled	Anti-inflammatory, anti-fibrotic, antiviral	COVID-19 ARDS	Phase 2	Completed	[92]
FP-1201 (Interferon β-1α)	Intravenous	Anti-inflammatory, anti-fibrotic, antiviral	Moderate-to-severe ARDS	Phase 3	Completed	[93]
GM-CSF	Intravenous	Immunomodulatory	not specified	Phase 2	Completed	[105]
rhGM-CSF	Inhaled	Immunomodulatory	Pneumonia	Phase 2	Active	NCT02595060
Molgramostim (rhGM-CSF)	Inhaled	Immunomodulatory	COVID-19 induced	Phase 2	Recruiting	NCT04569877
ANTICOAGULANTS AND FIBRINOLYTICS						
ALT-836	Intravenous	Anti-TF	ARDS patients requiring mechanical ventilation	Phase 1	Completed	[123]
ALT-836	Intravenous	Anti-TF	Sepsis-induced ARDS	Phase 2	Completed	NCT00879606
Tifacogin	Intravenous	Recombinant TFPI, modulates extrinsic coagulation pathway	Pneumonia	Phase 3	Completed	[125]
Antithrombin	Intravenous	Inhibition procoagulant thrombin	Sepsis-induced ARDS	Phase 3	Completed	[128, 129]
Heparin	Inhaled	Anticoagulant	ARDS patients requiring mechanical ventilation	Phase 1	Completed	[133, 134]
Heparin	Inhaled	Anticoagulant	ARDS patients requiring mechanical ventilation	Phase 2	Completed	[135]
Heparin	Inhaled	Anticoagulant	ARDS patients requiring mechanical ventilation	Phase 3	Completed	[136]
Heparin	Inhaled	Anticoagulant	Inhalation trauma ARDS	Not Applicable	Completed	[137]
Heparin	Inhaled	Anticoagulant	COVID-19 ARDS patients requiring mechanical ventilation	Phase 2/3	Recruiting	NCT04545541
ART-123	Intravenous	Cleaving protein C	Sepsis-induced	Phase 2b	Completed	[138]
Rh thrombomodulin and sivelestat	Intravenous	Cleaving protein C	ARDS and disseminated intravascular coagulation	Not Applicable	Completed	[139]
ART-123	Intravenous	Cleaving protein C	Sepsis-induced ARDS, ICU	Phase 3	Completed	[140, 141]
Drotrecogin alfa	Inhaled	rh Protein C	not specified	Not Applicable	Completed	[142]

T A B L E 1. Continued.

Drotrecogin alfa	Intravenous	rh Protein C	Sepsis-induced ARDS	Phase 3	Completed	[143]
Streptokinase	Nebulized	Conversion plasminogen to plasmin	Severe	Phase 3	Completed	[144]
ASPIRIN						
Aspirin	Oral	Cyclooxygenase pathway inhibitor	not specified	Phase 2b	Completed	[145]
Aspirin	Oral	Cyclooxygenase pathway inhibitor	ARDS patients requiring mechanical ventilation	Phase 2	Terminated	NCT02326350
OTHERS						
Alpha-1 antitrypsin	Intravenous	Serine protease inhibitor	COVID-19 ARDS	Not applicable	Completed	[147]
TRPV4 inhibitor	Inhaled	Decreasing inflammation	Healthy volunteers	Phase 1	Terminated	NCT03511105
FP-025		Inhibits MMP12	COVID-19 ARDS	Phase 2/3	Recruiting	NCT04750278
Sevoflurane	Inhaled	Anesthetic, anti-inflammatory	COVID-19 ARDS	Not applicable	Completed	[153]
Sevoflurane	Inhaled/ Intravenous	Anesthetic, anti-inflammatory	COVID-19 ARDS	Phase 3	Completed, unpublished	NCT04355962
Sevoflurane	Inhaled	Anesthetic, anti-inflammatory	Moderate-to-severe ARDS	Phase 3	Not yet recruiting	NCT04530188

ACE2: angiotensin converting enzyme 2; ALT-836: anti-TF antibody; ARDS: Acute Respiratory Distress Syndrome; ART-123: thrombomodulin alfa; ATII: alveolar type II epithelial cell; CD362: cluster of differentiation 362; cGMP: cyclic guanosine monophosphate; CO: carbon monoxide; COVID-19: Coronavirus Disease 2019; EPO: erythropoietin; FP-025: MMP12 inhibitor; FP-1201: recombinant human interferon-β; GM-CSF: granulocyte-macrophage colony-stimulating factor; GTP: guanosine-5'-triphosphate; hKGF: human keratinocyte growth factor; HMG-CoA: 3-hydroxy-3-methylglutaryl coenzyme A; hMSC: mesenchymal stem cell; ICU: intensive care unit; IL-6: interleukin-6; LPS: lipopolysaccharide; MMP12: matrix metallopeptidase 12; NETs: neutrophil extracellular traps; NLRP3: NOD-like receptor family pyrin domain containing 3; NOS: nitric oxide synthase; P39MAPK: P39 mitogen-activated protein kinase; RAS: rat sarcoma virus; rh: recombinant human; sGC: soluble guanylyl cyclase; SNG001: interferon-β drug; TFPI: tissue factor pathway inhibitor; Th2: T helper cell type 2; TNF: tumor necrosis factor; TNRF1: tumor necrosis factor receptor 1; Treg: T regulatory cell; TRPV4: transient receptor potential vanilloid 4; VEGF: vascular endothelial growth factor.

of mechanical ventilation nor physiologic function [17]. However, in a randomized controlled phase 3 trial intratracheal recombinant surfactant protein C in patients with ARDS from various etiologies did improve gas exchange but not survival [18, 19]. In a post hoc analysis, recombinant surfactant protein C proved to decrease mortality in patients with ARDS due to pneumonia or aspiration [20].

Surfactant replacement has also been proposed for COVID-19. In a retrospective analysis, poractant alfa (Curosurf), a surfactant replacement therapy, administered through a bronchoscopy, proved to be safe and produce a non-significant 28 days mortality reduction in adult COVID-19-ARDS patients [21]. Presently, phase 2 studies to evaluate efficacy and safety of three poractant alfa (Curosurf) administrations by endotracheal instillation every 24 h, or 3 mL/kg of poractant alpha (Curosurf) administered by bronchial fibroscopy in adult ARDS patients due to COVID-19 are being conducted (NCT04502433 and NCT04384731).

2.2.2 AP301

Transepithelial ion transport is affected by alveolar epithelium injury, which impairs excess liquid removal from the alveolar space [22]. AP301 (Solnatide) is a synthetic peptide that has been proved to activate alveolar epithelium sodium channels [23].

In a phase 2a randomized controlled trial inhaled AP301 every 12 h for 7 days in patients with ARDS requiring mechanical ventilation decreased extravascular lung water and ventilation pressures over 7 days in patients with Sepsis related Organ Failure Assessment (SOFA) scores ≥11 [24, 25]. Currently, there is a phase 2b randomized controlled dose-escalation study to determine the safety of multiple ascending doses (5 mg, 60 mg, 125 mg) inhaled every 12 h through 7 days in patients with moderate-to-severe ARDS (NCT03567577) [26].

2.2.3 Keratinocyte Growth Factor

Keratinocyte Growth Factor (KGF) is an epithelial growth factor that induces ATII cells proliferation and promotes migration

and regeneration of the alveolar epithelium. Because of its action on ATII cells, KFG also maintains ionic transport and surfactant functions of ATII cells [27]. In preclinical models of acute lung injury, KGF decreased infiltration of neutrophils in the alveoar space, edema, permeability and epithelial injury [28].

In the phase 2 of keratinocyte growth factor for the treatment of the ARDS (KARE) randomised clinical trial, intravenous palifermin, a recombinant human KGF, did not ameliorate physiological nor clinical outcomes in patients with ARDS. Although the study was not powered to assess ventilation and mortality, those were higher in patients that received palifermin [29]. Authors recommended not to use KGF to treat ARDS patients, however they also specified that the study was performed in a heterogeneous population regarding ARDS etiology, and that focus KGF therapy on an ARDS subphenotype might be a better option to determine KGF response.

2.3 Alveolar endothelium

The alveolar endothelium is exposed to higher oxygen tensions while maintaining low-pressure blood flow compared to the systemic vascular endothelium. When there is a damage, injured alveolar endothelium promotes the destruction of the vascular bed and the expression of proinflammatory, reactive oxygen species and recruitment molecules, together with enhanced procoagulant activity and clot formation [14].

2.3.1 Nitric Oxide Synthase

Citrulline is the substrate of nitric oxide synthase (NOS) and lower levels are linked to decreased functional gut mass [30]. A randomized phase 2 study of intravenous citrulline revealed no effectivity in ARDS patients with severe sepsis, although the completion of the study has not still been published (NCT01474863). Another randomized trial with dietary enterally L-citrulline administration in patients with COVID-19-ARDS has finished and the results have to be published (NCT04404426).

After the conversion of arginine into citrulline, the NOS produces the gas nitric oxide (NO). Inhaled NO has been demonstrated to improve oxygenation but does not reduce mortality and might be harmful in 14 randomized controlled trials in adults with ARDS [31].

NO activates soluble guanylate cyclase (sGC), which converts GTP into cGMP. Oxidative stress decreases the NO-sGC-cGMP pathway with sGC inactivation. The therapeutic use of sGC modulators is centered on ameliorations in alveolar and vascular development of premature neonatal lungs not properly developed [32]. In a chronic hypoxia-induced newborn rat model, the administration of BAY41-2272 (sGC-cGMP stimulator) or sildenafil (cGMP-specific phosphodiesterase 5 inhibitor) results in pulmonary vascular resistance, which is reduced when those treatments are combined [33]. Presently, in a phase 1 clinical study multiple doses (three times a day for a week) of BAY1211163 by inhalation are being administered in patients with ARDS, in order to determine the safest dose (NCT04609943).

2.3.2 Prostacyclin

Iloprost is a synthetic analogue of prostacyclin and its aerosolization results in selective pulmonary vasodilatation. A randomized phase 2 clinical trial with inhaled iloprost for 5 days in ARDS patients is being conducted (ThIlo) (NCT03111212) [34]. Concerning COVID-19, a phase 2 randomized controlled trials with inhaled epoprostenol in severe patients with COVID-19 (VPCOVID) (NCT04452669) was presently completed although results have not still been published, and a phase 2 randomized clinical trial with iloprost in COVID-19 patients (ILOCOVID) (NCT04445246) is being performed.

2.3.3 Anti-Vascular Endothelial Growth Factor

Vascular endothelial growth factor (VEGF) increases lung vascular permeability [35]. In a preclinical model of increased permeability and pulmonary edema in mice, Bevacizumab (anti-VEGF) histological analysis revealed reduced edema fluid, decreased lung wet-to-dry ratio and bronchoalveolar lavage protein levels [36]. A phase 2 clinical trial with a single intravenous bevacizumab administration in patients with severe sepsis was withdrawn due to underfunding (NCT01314066). Nevertheless, in two cases of COVID-19 induced atypical pneumonia, Bevacizumab ameliorated patients outcome [37]. A phase 2 study with intravenous 500 mg Bevacizumab in patients with severe COVID-19 was just completed but results have not been published yet (NCT04275414).

2.3.4 Levosimendan

Levosimendan is a calcium sensitizer that opens adenosine triphosphate-dependent potassium channels with vasodilator effects [38]. A randomized phase 3 study with 0.5 mL/h of levosimendan in patients with ARDS is being conducted (NCT04020003). In a randomized controlled pilot study levosimendan ameliorates right ventricular performance and pulmonary vasodilator effect in septic patients with ARDS [39]. Secondary analysis of randomized controlled trials in septic patients reveal that the survival of the levosimendan group was lower [40].

3. Mucolytics

The respiratory tract contains secretions composed by mucin glycoproteins, but in patients with respiratory diseases the mucus presents a higher viscosity. N-acetylcysteine is an antioxidant derived from the amino acid cysteine and is the most widely recommended mucolytic. In a randomized clinical trial 150 mg/kg of N-acetylcysteine produced a significant difference in the consciousness of ARDS patients requiring mechanical ventilation [41]. A pilot study of intravenous N-Acetylcysteine in patients with mild-to-moderate COVID-19 did not prove benefit [42].

Neutrophil extracellular traps (NETs) and damage-associated molecular patterns (DAMPs) resulting from the inflammatory response contain extracellular DNA among other compounds [43]. Dornase alfa is a recombinant human Deoxyribonuclease (DNAse 1) commonly used in the treatment of cystic fibrosis. It acts as a mucolytic by cleaving

extracellular DNA, thereby facilitating airway clearance and reducing alveolar hyper-inflammation [44]. The terminated phase 3 COVIDornase study (NCT04355364) and another phase 2 study (NCT04402944) in recruitment stage propose inhaled Dornase alfa therapy for ventilated patients with COVID-19-related ARDS.

4. Bronchodilators

Beta-adrenergic agonists ($\beta2$ agonists) have a beneficial effect in alveolar fluid clearance and permeability. Salbutamol is a beta-adrenergic agonist. In a randomized controlled trial intravenous salbutamol for 7 days decreased extravascular lung water in patients with ARDS requiring mechanical ventilation [45]. However, in a randomized phase 2 trial intravenous salbutamol for up to 7 years was poorly tolerated and did not present benefit in patients with ARDS [46]. In another randomized phase 2 clinical trial, intravenous salbutamol early in the development of ARDS was not safe [47].

Nebulized bronchodilators have also been proposed. In randomized clinical trial nebulized albuterol did not improve clinical outcomes in patients with ARDS [48]. A clinical trial with nebulized Dornase Alfa co-administered with abuterol in patients with COVID-19 requiring mechanical ventilation has just been completed but results have not still been announced (NCT04387786). Also, there is an ongoing phase 1 study comparing nebulized lidocaine, salbutamol and beclomethasone plus salbutamol in patients with COVID-19-ARDS and non-invasive ventilation (NCT04979923).

5. Immunomodulation

5.1 Neuromuscular blockers

The neuromuscular transmission is blocked by neuromuscular blocking agents at the neuromuscular junction, in order to minimize volutrauma, ventilator-induced lung injury, and biotrauma [49].

In a multicenter randomized trial, the early administration of neuromuscular-blocking agent cisatracurium in patients with moderate to severe ARDS improved 90-day survival and the time off the ventilator [50]. However, in another clinical trial, and early and continuous infusion of cisatracurim did not decrease 90-day mortality in patients with moderate-to-severe ARDS [51]. Current evidence favors avoiding a continuous infusion of neuromuscular blockers in patients with mechanical ventilation but use a lighter sedation strategy, and for patients who need a deep sedation to facilitate lung protective ventilation or prone positioning, to infuse neuromuscular blockers for 48 h is a reasonable option [52].

5.2 Steroids

Steroids are powerful anti-inflammatory and anti-fibrotic drugs that may lead to high-risk infections due to the suppression they exert on the immune system.

Clinical trials suggest that steroid treatment in ARDS patients would be indicated at the onset of the pathology. Administered corticosteroids 72 h after ARDS diagnosis decreased lung damage and increased ventilator weaning [53]. A meta-analysis in ARDS patients concluded that low-dose corticosteroids in early ARDS significantly reduced mortality and the duration of mechanical ventilation, whereas high doses did not [54]. A different meta-analysis shows that steroid treatment improves mortality and promotes shorter ventilation periods [55]. In contrast, in patients with influenza pneumonia, the early use of steroid therapy is associated with increased mortality [56, 57]. Nonetheless, studies in patients with community-acquired pneumonia treated with corticosteroids showed a reduced risk of treatment failure [58], reduced mortality, hospital stay and need for mechanical ventilation [59].

Dexamethasone is one of the most clinically used steroids for treatment. In a phase 2/3 trial patients with moderate-to-severe ARDS requiring mechanical ventilation were intravenously administered with dexamethasone (20 mg for 5 days, then 10 mg for the next 5 days) and presented an increase in the number of ventilator-free days and reduced mortality [60].

In COVID-19 patients, treatment with dexamethasone (intravenous or oral, 6 mg/day for 10 days) resulted in a lower incidence of death in those patients requiring invasive mechanical ventilation compared to those not requiring ventilator support [61]. Some of the clinical trials now recruiting are the phase 4 REMED study (NCT04663555), which aims to test two different doses (6 mg *vs.* 20 mg) of intravenous dexamethasone in SARS-CoV-2-induced ARDS patients. Or a phase 3 study that aims to compare intravenous treatment with dexamethasone or methylprednisolone in COVID-19 patients with ARDS (NCT04499313).

Regarding hydrocortisone, in a trial, patients with ARDS-associated sepsis were treated with a dose of 50 mg every 6 h within 12 h of their ARDS diagnosis. The treated group showed improvements in pulmonary physiology, but not a decrease in mortality compared to the placebo group [62].

Another of the most investigated corticosteroids for future therapies is methylprednisolone. In 24 patients with severe ARDS methylprednisolone (2 mg/kg/day for 32 days) decreased in-hospital and ICU mortality [63]. In the first 72 h, patients with ARDS were treated with an infusion of methylprednisolone (1 mg/kg/day) for 28 days, and had decreased C-reactive protein, mechanical ventilation and mortality [64]. A phase 2 study proposed intrapleural administration of the steroid Solumedrol (methylprednisolone) versus conventional treatment with extracorporeal membrane oxygenation and intravenous steroid administration. Results are not yet available (NCT01423864).

The MINECRAFT phase 2 study, aims to study the efficacy of administering canrenone, a steroidal antimineralocorticoid, intravenously in moderate-to-severe ARDS patients due to SARS-CoV-2 infection (NCT04977960).

A recently explored field is the administration of inhaled steroids. Early treatment consisting of inhaled budesonide together with a beta-agonist in patients at risk of developing ARDS improved oxygenation [65]. Another study where nebulised budesonide was administered also improved oxygenation and reduced proinflammatory cytokines (Tumor necrosis factor-α (TNF-α), Interleukin (IL)-1β and IL-6) [66]. There is a phase 2 study in paediatric ARDS patients with inhaled budesonide (NCT04064684). In neonatal patients with severe ARDS requiring mechanical ventilation, intratracheal treat-

ment with budesonide and surfactant resulted in a decreased incidence of bronchial dysplasia or death and decreased inflammation [67]. In children on mechanical respiratory support, treatment with budesonide and surfactant did not improve survival or the development of bronchial dysplasia over the surfactant-treated group but decreased the need for mechanical ventilation [68].

5.3 Statins

Statins are β-Hydroxy β-methylglutaryl-CoA (HMG-CoA) reductase inhibitors with immunomodulatory properties. A meta-analysis showed that treatment with statins prior to intensive care unit (ICU) admission or before a diagnosis of a specific pathology showed a decrease in 30-day mortality, but no association with in-hospital mortality [69].

The Hydroxymethylglutaryl-CoA reductase inhibition with simvastatin in Acute lung injury to Reduce Pulmonary dysfunction (HARP-2) trial was a multicentre trial that sought to test simvastatin (80 mg/day) in ICU patients, 48 h after the onset of ARDS. Patients involved in the study could be divided into two different sub-phenotypes: hypo-inflammatory (65%) and hyper-inflammatory (35%), and only increased survival was found in patients who had a hyper-inflammatory subphenotype treated with simvastatin. This study highlighted the need to phenotype different types of ARDS patients [70]. In the Statins for Acutely Injured Lungs from Sepsis (SAILS) trial they were also able to identify different biological phenotypes but did not see phenotype-specific benefit from rosuvastatin treatment [71]. In recent years, clinical studies propose to investigate the role of statins in ARDS patients of different aetiologies, although those have been cancelled due to lack of enrolment or other causes.

5.4 Carbon monoxide

Carbon monoxide (CO) results from the catabolism of heme oxygenase within the body. Its anti-inflammatory and anti-apoptotic role has been described. CO down-regulates the NOD-like receptor family pyrin domain containing 3 (NLRP3) inflammasome, thus preventing mitochondrial dysfunction, and protects against cellular oxidative stress in models of lung injury [72, 73]. In *in vivo* models that received lypopolysaccharide (LPS), 50 parts per million (ppm) inhaled CO restored arterial resistance and decreased NOS-2 expression, although no changes were seen in plasma levels of inflammatory cytokines [74]. In a nonhuman primate pneumonia model CO treatment (200 ppm of concentration for 60 minutes) reduced extravascular alveolar fluid [75].

In a phase 1 trial in patients with ARDS-induced sepsis, a low dose (100–200 ppm) of inhaled CO was found to be a well-tolerated and safe treatment during mechanical ventilation. A phase 2 trial is currently recruiting ARDS patients to be treated with inhaled carbon monoxide at 200 ppm (NCT03799874).

5.5 Mesenchymal Stromal Cells

Mesenchymal Stromal Cells (MSCs) have immunomodulatory properties and reparative effects on damaged tissue, presenting paracrine and cell-cell communication (see chapter Cell Ther-

apies in ARDS). Their role as a treatment in ARDS depends on the microenvironment to which the cell therapy is exposed and what has caused the lung injury [76].

The phase 1/2 clinical trial MultiStem Therapy in ARDS (MUST-ARDS) evaluated the safety of intravenous 900 million bone marrow-derived multipotent adult progenitor cells administered within 96 h of the onset of moderate-to-severe ARDS patients requiring mechanical ventilation. Administration of the cells was well tolerated and tended to decrease the need for mechanical ventilation [77].

In the Human Mesenchymal Stromal Cells for ARDS (START) phase 2a trial, patients with moderate-to-severe ARDS requiring mechanical ventilation were given an intravenous dose of MSCs, which was safe but showed no improvement over the placebo-treated group. These findings have been attributed to the low viability of the administered cells [78]. The Mesenchymal Stromal Cells for ARDS (STAT) phase 2b trial, an extension currently recruiting, aims to test the safety and efficacy of 10 million MSCs/kg (NCT03818854).

The REALIST trial proposes to investigate whether a single infusion of MSCs (human umbilical cord-derived CD362 enriched MSCs) could help in the treatment of ARDS, a phase 1/2 study (NCT03042143).

Regarding COVID-19-induced ARDS, it has been shown that in 7 patients who received a transfusion of ACE2$^-$ MSCs, lung function and symptomatology improved two days after treatment, and inflammation was reduced by decreasing C reactive protein (CRP) and TNF-α [79].

5.6 Regulatory T-cells

Regulatory T-cells (Treg cells) act on the immune system by decreasing its activation and promoting homeostasis. Overexpression of Transforming Growth Factor (TGF) $\beta 1$, the most secreted cytokine by Treg cells, in a murine model of acute lung injury (ALI) induces more Treg cells and decreases T helper 17 cells (Th17) cells, improving lung inflammation [80].

Several clinical trials are currently ongoing in COVID-19-ARDS patients proposing intravenous administration of Treg cells (NCT05027815 and NCT04468971), and a study in COVID-19-ARDS patients receiving intravenous Treg/Th2 hybrid cells has just been terminated, although results are not posted yet (NCT04482699).

5.7 Vitamin C

Vitamin C is an antioxidant molecule with protective effects. In one study, vitamin C levels were found to be undetectable in more than 90% of patients with SARS-CoV-2-associated ARDS [81]. In the Vitamin C in patients with Sepsis and Severe Acute Respiratory Failure (CITRIS-ALI) phase 2 trial, patients with sepsis and consequent ARDS were treated with a 96h-infusion of vitamin C. There was no difference between the vitamin C-treated group and the control group in terms of decreased inflammation, but secondary outcomes showed a decrease in 28-day mortality in the treated group [82].

Completed but unpublished clinical studies include COVID-19 patients with ARDS treated with vitamin C and other antiox-

idants (NCT04570254), and ascorbic acid (NCT04710329). Also, there is a phase 3 study in septic patients with ARDS that proposes to compare the effect of high-dose intravenous vitamin C, but is not yet enrolling patients (NCT04404387).

5.8 Ulinastatin

Ulinastatin, a glycoprotein known as urinary trypsin inhibitor, is an experimental drug with anti-inflammatory properties. A clinical study of 14 consecutive days of treatment with ulinastatin in ARDS patients requiring mechanical ventilation resulted in decreased TNF-α, IL-6 and CRP levels, increased antioxidant capacity, decreased ventilatory need and hospital-stay days [83].

5.9 Inhibitors

5.9.1 p38

The p38 mitogen-activated protein kinases (p38MAPK) are intracellular signals that play a crucial role in igniting inflammation through the release of proinflammatory cytokines such as IL-6, IL-1β and TNF-α [84].

In patients at risk of developing ARDS, a phase 2 study using dilmapimod, a specific inhibitor of p38MAPK, was shown to be well tolerated, with the highest dose (10 mg) administered as a continuous infusion over 24 h having the most favourable profiles and decreasing IL-6 and CRP [84].

The hyper-inflammatory response that occurs in SARS-CoV-2 infection may be caused by up-regulation of p38MAPK activity [85]. SARS-CoV-2 has previously been shown to act on the p38MAPK pathway, promoting inflammation, vasoconstriction and thrombosis and in turn favouring the continuation of the viral cycle. A preclinical study in which a p38 inhibitor was administered to SARS-CoV-infected mice showed an 80% survival rate in the treated group [86]. Among the proposed inhibitors, losmapimod is one of the most clinically studied inhibitors [85].

5.9.2 Tumor Necrosis Factor Receptor 1

Another pathway antagonised has been the TNF-α pathway, mainly by an anti-TNF-1 receptor (TNFR1) antibody that selectively binds to the TNFR1.

TNFR1 and TNFR2 levels are elevated in patients with critical COVID-19. In addition, markers of monocyte activation such as soluble cluster of differentiation 14 (sCD14) have been found to be directly correlated with TNFR1, suggesting an association with severe disease, and might be predictive for mortality in critically ill patients. The TNF/TNFR signalling pathway is an interesting target to improve survival in COVID-19 critical patients [87]. In healthy humans previously administered LPS, anti-TNFR1 treatment resulted in decreased inflammatory response, endothelial damage, and neutrophil infiltration into the lung [88].

5.9.3 Interleukin-6

IL-6 is secreted by T cells contributing to inflammation [89], which ends up in an increased ARDS pathophysiology. The administration of IL-6 blockers tocilizumab and sarilumab proved benefit in patients with ARDS [90]. Phase 2/3 clinical trials of intravenous tocilizumab in COVID-19-ARDS patients have been completed, but results have not still been published (NCT04445272), and other clinical trials are recruiting (NCT04412772, NCT05082714).

5.9.4 Interferons

Interferons comprise a set of molecules with different functions that may have opposing roles in ARDS. Interferon-γ (IFNγ) is notably involved in viral infections, being highly proinflammatory. In COVID-19 patients who develop ARDS, treatment with anti-IFNγ could be a potential treatment, since IFNγ has been observed to upregulate ACE2 expression in the lung epithelium, a receptor used by SARS-CoV-2 for cell entry [91].

On the contrary, interferon β-1α has anti-inflammatory, anti-fibrotic and antiviral properties. In a phase 2 trial Interferon β-1α (SNG001), nebulised inhaled interferon β-1α was administered to COVID-19 patients and proved a fast recovery from infection. It has also been recommended to test interferon β-1α in ventilated and critically ill patients [92]. In contrast, in a phase 3 study in patients diagnosed with moderate-to-severe ARDS, intravenous administration of FP-1201 (a recombinant human interferon β-1α), showed no improvement compared to placebo administration [93].

5.9.5 Imatinib

Imatinib, a tyrosine kinase inhibitor, attenuates oxidative damage by acting on lung endothelial catalase. Imatinib has been shown to attenuate ALI in preclinical double hit models (LPS- and ventilator-induced lung injury or VILI) [94] and to decrease mortality in models where intravenous LPS was administered [95].

In a phase 1 study, healthy individuals were treated orally with imatinib and then given inhaled LPS (NCT03328117). The results have not yet been published.

In silico studies have proposed imatinib as promising therapy for SARS-CoV-2 infection [96]. There is currently a phase 3 study enrolling hospitalised COVID-19 patients, which aims to evaluate the efficacy and safety of oral administration of imatinib [97].

5.9.6 NLRP-3 Inhibitors

The multiprotein cytosolic complex composed of NLRP3 oligomerization forms an inflammasome that causes the release of proinflammatory cytokines such as IL-1β and IL-18 through a dependent-caspase-1 mechanism.

In *in vitro* studies, the NLRP3 inflammasome inhibitor pirfenidone inhibited NLRP action by suppressing reactive oxygen species (ROS) generation. Furthermore, in a murine model instilled intratracheally with LPS, oral administration of pirfenidone mitigated lung inflammation and fibrosis [98].

Nowadays, there is a phase 3 study where pirfenidone is administered orally to COVID-19 patients (NCT04282902) and another in COVID-19 patients with severe ARDS, where it is administered through a nasogastric tube (NCT04653831).

Tetracycline, another NLRP3 inflammasome inhibitor, administered intraperitoneally in murine models has reduced mortality, lung injury and IL-1β concentration compared to those treated with phosphate-buffered saline (PBS) [99]. A

clinical trial is recruiting ARDS patients to evaluate the inhibition of human leukocyte immune response treated with tetracycline (NCT04079426).

A recent study in a murine ALI model has shown that intraperitoneal administration of erythropoietin (EPO) suppresses the NLRP3 inflammasome by inhibiting the Nuclear Factor kappa B (NF-κB) cellular pathway and consequently decreasing lung damage [100]. A phase 2 trial is studying a therapy with Vadadustat, a Hypoxia inducible factor prolyl-hydroxylase inhibitor drug that increases endogenous EPO production, in hospitalised COVID-19 patients with ARDS (NCT04478071).

5.9.7 Granulocyte-macrophage colony-stimulating factor

Granulocyte-macrophage colony-stimulating factor (GM-CSF) is an immunomodulatory cytokine that has an important role in inflammation but has also been described to be crucial in antimicrobial defence in the lung and in surfactant homeostasis [101, 102]. More research is needed to define the role of cytokine in the course of ARDS.

In animal models, administration of GM-CSF proved benefit to the epithelium, restoring tissue homeostasis and barrier function, and limiting hyperoxic lung injury [103, 104]. In a randomised phase 2 trial of patients with ARDS GM-CSF infusion did not increase the number of ventilator-free days nor reduce mortality [105].

Inhaled GM-CSF administration might have improvements in the severity of ARDS [106]. A phase 2 study in patients with ARDS-associated pneumonia tested inhaled administration of a recombinant human GM-CSF, with no available results yet (NCT02595060). Now, the phase 2 GI-COVID study (NCT04569877) is recruiting COVID-19 patients to administer a nebulised solution of molgramostim (a human recombinant GM-CSF).

In contrast, GM-CSF polarises myeloid cells towards a proinflammatory phenotype and therefore it has been proposed to block its signalling. Due to the hyper-inflammatory situation in SARS-CoV-2 infection, clinical studies with anti-GM-CSF antibodies have been conducted in these patients. A phase 2 study with Otilimab (NCT04376684) and a phase 3 study with Lenzilumab (NCT04351152) have been carried out, although no results have been published yet. Others trials that propose anti-GM-CSF as a therapy for COVID-19 patients are recruiting right now (NCT04341116, NCT04400929). The Mavrilimumab in patients with severe COVID-19 pneumonia and systemic hyperinflammation (MASH-COVID) study showed that intravenous administration of mavrilimumab (anti-GM-CSF) showed no significant difference in survival compared to placebo [107]. The realisation of randomised trials will be essential to define the therapeutic effect of GM-CSF blockade in ARDS and COVID-19 [108].

5.9.8 Neutrophil-proteases inhibitors

In ARDS, neutrophils can promote cell damage through oxidative stress, the release of NETs and secretion of proteases [109].

Silvelestat is an inhibitor of neutrophil elastase, which has proteolytic activity and induces the production of inflamma-

tory cytokines [110, 111]. The use of Silvelestat has been shown not to affect the bactericidal capabilities of neutrophils [112]. After several preclinical studies in which it has been shown to reduce mortality and parameters such as vascular permeability and inflammation [113–115], clinical studies aim to determine its protective role in ARDS. Favourable results have been observed in patients with mild ARDS [116], while another study showed no effect on 28–30 days mortality and ICU stays [117]. A phase 3 study in ARDS patients with sepsis (NCT04973670) and a phase 4 multicenter clinical trial in ARDS patients with systemic inflammatory response syndrome (NCT04909697) are ongoing.

Elafin is an endogenous protease inhibitor. The imbalance between neutrophil elastase and elafin is associated with mortality in ARDS [118]. In an LPS-induced mouse model of ALI, instillation of a cleavage-resistant variant of elafin (GG-elafin) was able to decrease neutrophil-induced inflammation as well as decrease protease activity compared to wild-type elafin [119].

6. Anticoagulants and Fibrinolytics

A major hallmark of ARDS is deregulated coagulation and fibrinolysis, leading to pulmonary coagulopathy in ARDS and systemically altered coagulation in septic patients [120, 121]. Coagulation and inflammation play an essential role in ARDS. Given the close interactions between these systems [122], anticoagulants might act on ARDS pathophysiology because of their anticoagulant and anti-inflammatory activities.

6.1 Tissue Factor

Tissue Factor (TF) is a transmembrane protein that is the major initiator of the extrinsic coagulation pathway when activated by the binding of factor VIIa. ALT-836 is an anti-TF antibody that blocks the binding of factor VIIa, and a single intravenous dose of ALT-836 (0.06, 0.08, 0.1 mg/kg) has proved to be safe in a randomized controlled phase 1 trial in patients with ARDS requiring mechanical ventilation [123]. A randomized phase 2 clinical trial in patients with sepsis and ARDS receiving a single (0.06 mg/kg) intravenous dose up to four doses has already been performed, although results have not been published yet (NCT00879606).

Tissue Factor Pathway Inhibitor (TFPI) modulates the initiation of the extrinsic coagulation pathway. A randomized controlled phase 3 clinical trial of intravenous tifacogin (recombinant TFPI) administration during 96 h in patients with severe sepsis did not reduce mortality and was associated with increased bleeding [124]. However, in a randomized controlled phase 3 clinical trial intravenous tifacogin administration during 96 h did not decrease mortality but reduced prothrombin fragment and thrombin antithrombin complexes levels in patients with severe community-acquired pneumonia [125].

6.2 Antithrombin

Antithrombin is a serine protease inhibitor synthesized in the liver [126, 127], and is known to inhibit procoagulant enzymes including thrombin, factor Xa, IXa, XIa and XIIa. When

heparin binds to antithrombin, its inhibitory activity is 1000-fold increased [127].

A randomized phase 3 clinical trial with intravenous 30,000 IU of antithrombin within 4 days had no effect on mortality in patients with severe sepsis (the KyberSept Trial), although an increased risk of hemorrage was detected when administering antithrombin and heparin together [128]. A post hoc analysis in patients with severe sepsis treated in a single center early after onset revealed increased bleeding due to antithrombin [129].

Nebulized antithrombin alone or combined with heparin attenuated lung injury in HCl/LPS-induced ALI in rats, reducing pulmonary coagulation and inflammation without altering systemic coagulation nor bleeding [130].

6.3 Heparin

Heparin is a natural anticoagulant produced by mast cells in the intestine or lungs, basophils in the blood and endothelial cells [131]. Heparin presents anticoagulant actions potentiating antithrombin inhibitory activity and enhancing TFPI, and anti-inflammatory actions both related or not to thrombin inhibition [132].

Controversial results have been determined in patients with ARDS while administering local heparin. In a phase 1 trial nebulized heparin (50,000 IU/day, 100,000 IU/day, 200,000 IU/day, 400,000 IU/day) did not produce adverse effects and attenuated pulmonary coagulopathy in patients with ARDS requiring mechanical ventilation [133, 134], and in a randomized phase 2 study nebulized heparin (25,000 IU) reduced days of mechanical ventilation in patients with ARDS [135]. Also, in a randomized phase 3 clinical trial (CHARLI) nebulized heparin (250,000 IU) every 6 h to day 10 was well tolerated with decreased lung injury progression and earlier return at home in patients with invasive ventilation [136]. In contrast, in a randomized controlled trial with nebulized heparin focused on the safety and efficacy of burn patients with inhalation trauma (HEPBURN), the trial was stopped because of increased systemic clotting times and adverse events [137].

Nebulized heparin has also been proposed for COVID-19 patients. A randomized phase 2/3 clinical trial with nebulized 25,000 IU of heparin every 6 h for up to 10 days in patients with COVID-19 requiring mechanical ventilation is being performed (NCT04545541).

6.4 Thrombomodulin

The Protein C Pathway also has a major role in coagulation and fibrinolysis regulation. Thrombomodulin is a thrombin receptor, and, when the complex is formed, protein C is cleaved and activated protein C is produced.

ART-123 is a recombinant human soluble thrombomodulin. In a randomized controlled phase 2b study intravenously administered ART-123 for 6 days proved to be safe and effective reducing prothrombin fragment and thrombin-antithrombin complex concentrations in patients with sepsis-associated disseminated intravascular coagulation [138]. In addition, in a retrospective study intravenously combined sivelestat and recombinant human soluble thrombomodulin improved 60-day survival and ventilator-free days in patients with ARDS and disseminated intravascular coagulation [139].

In the full analysis of the phase 3 multinational Scarlet study that evaluate the efficacy and safety of intravenous ART-123 during 6 days to treat sepsis-associated coagulopathy, no statistically differences were determined [140]. However, in post hoc analysis in patients with sepsis-associated coagulopathy that did not receive concomitant heparin, ART-123 proved more benefit, indicating that heparin administration could impact ART-123 efficacy, a fact that should be confirmed in further studies [141].

6.5 Activated Protein C

Alveolar epithelial cells release thrombomodulin from the cell surface, due to a metalloproteolytic process, and this decreases the ability of these cells to activate Protein C [120, 121].

Inhaled drotrecogin alfa (recombinant human activated Protein C) in patients with ARDS decreased coagulation, neutrophils recruitment and inflammation in the alveolar compartment and increased fibrinolysis without producing systemic effects [142]. However, in a randomized multicentre phase 3 Prowess-Shock trial intravenous drotrecogin alfa (recombinant human activated Protein C) in 1967 patients with septic shock did not reduce mortality [143], and no further studies have been performed becaause Activated Protein C was removed from the market.

6.6 Streptokinase

Plasminogen activator and inhibitor pathway regulate fibrin deposition. Streptokinase binds plasminogen and drives the conversion of plasminogen to plasmin, a fibrinolytic enzyme. In a randomized phase 3 trial nebulized streptokinase in patients with severe ARDS improved oxygenation and lung mechanics [144].

7. Aspirin

Coagulation cascade activation leads to increased platelet recruitment and thrombin formation in the lung. Aspirin is a non-selective inhibitor of the cyclooxygenase pathway, with reduced platelet recruitment, fibrinolytic and decreased inflammatory effect. In a randomized controlled phase 2 trial aspirin did not decrease the risk of ARDS [145]. Also, a randomized phase 2 clinical trial with enterally 75 mg aspirin administration in patients with ARDS requiring mechanical ventilation is terminated (STAR), although results have not been announced (NCT02326350).

8. Others

8.1 Alpha-1 antitrypsin

Alpha-1 antitrypsin is a serine protease inhibitor that has been found to ameliorate oxygenation [146]. A randomized phase 2 clinical trial with intravenous prolastin (plasma-purified alpha-1 antitrypsin) in patients with COVID-19 ARDS is presently being conducted [147].

8.2 Transient Receptor Potential Vanilloid 4 inhibitor

The mechanosensitive cation calcium channel Transient Receptor Potential Vanilloid 4 inhibitor (TRPV4) is an essential homeostasis regulator that is implicated in ARDS inflammation [148]. TRPV4 can induce alveolar endothelial and epithelial dysfunction, which results in increased permeability and edema [149]. Nevertheless, in a preclinical model with intratracheal *Pseudomonas aeruginosa* in mice, TRPV4 activity has demonstrated to enhance macrophages phagocytosis and decrease inflammation [150]. Various TRPV4 inhibitors have proved to decrease acute lung injury in preclinical models. In mice exposed to hydrochloric acid or chlorine gas, TRPV4 inhibitor reduced inflammation and vascular leakage [151]. In a first clinical study, TRPV4 inhibitor did not produce ameliorations in healthy patients receiving inhaled LPS (NCT03511105).

8.3 Matrix metalloproteinase 12 inhibitor

FP-025 inhibits matrix metalloproteinase-12 (MMP12), an enzyme that degrades and remodels the extracellular matrix but is also known to modulate the influx of monocytes and macrophages in the alveolar compartment [152]. There is an ongoing randomized phase 2/3 clinical study with FP-025 (100 or 300 mg) in patients with severe and critical COVID-19-ARDS (NCT04750278).

8.4 Sevoflurane

Sedation with the volatile anesthetic sevoflurane-induced anti-inflammatory processes in ventilated patients [153]. In a randomized controlled phase 3 clinical trial volatile or intravenous sedation with sevoflurane for 48 h has been administered in patients with COVID-19-ARDS, although results have not been published yet (NCT04355962). Another randomized phase 3 trial to determine the effects of inhaled sevoflurane sedation on extravascular lung water and pulmonary vascular permeability in ARDS patients is proposed, although the recruitment has not started (NCT04530188).

9. Preclinical therapies for ARDS

9.1 Adenosine A2A receptor agonists

The nucleoside adenosine has anti-inflammatory properties, and its deficiency has been shown to increase pulmonary oedema and inflammation in a murine model of VILI [154].

Pharmacological intervention with the adenosine A2A receptor agonist CGS-21680 in rat VILI models reduced pulmonary edema, respiratory elastance and neutrophil recruitment into the lung compared to vehicle-treated animals [155]. In different models of ALI induced by HCl, LPS or *Escherichia coli*, instillation of the agonist GW328267C led to alveolar fluid clearance [156].

Blockade of equilibrative nucleoside transporters (ENTs) with dipyridamole increases adenosine in the alveolus and decreases pulmonary edema and improves gas exchange during ALI [157, 158].

9.2 Protease-activated receptor 1

Coagulation activates inflammation through protease-activated receptors (PARs) [159]. PAR1 is expressed in epithelial lung cells and the endothelium and is associated with a prothrombotic state [160, 161]. Thrombin binds to PAR1 and stimulates neutrophil recruitment and the release of proinflammatory cytokines [162]. Nevertheless, in antigen-presenting cells, PAR1 activation decreases the production of proinflammatory cytokines [163].

In influenza virus infection in mice, after activating PAR1 receptors with an agonist, they found increased lung inflammation but did not affect survival. They also observed that activated PAR1 increased the conversion of plasminogen to plasmin [164]. PAR1 antagonists are only in clinical trials for other pathologies.

9.3 Receptor for advanced glycation end-products inhibitors

The soluble receptor for advanced glycation end-products (sRAGE) is a marker of epithelial damage, especially in ATI, and is a prognostic marker for ARDS [165, 166].

Blockade of RAGE, using anti-RAGE antibody or sRAGE decoy receptor in acid-induced mice model of ALI reduced RAGE mRNA levels in the lung, restored alveolar-capillary barrier permeability after injury, decreased the total number of leukocytes in bronchoalveolar lavage (BAL) and restored membrane aquaporin-5 expression [167]. In piglets, blockade of RAGE decreased alveolar inflammation and induced alveolar fluid clearance [168]. A recent study in LPS-induced ALI murine model has shown that RAGE signalling mediates epithelial barrier dysfunction, enhancing lung inflammation and causing loss of adherent junctions [169].

9.4 Haptoglobin

Haptoglobin acts as a scavenger receptor for cell-free haemoglobin (CFH), which is elevated during sepsis and correlated with increased mortality. Haptoglobin decreases CFH levels and iron levels, leading to less oxidative damage to the lung in sepsis, but it has not been shown to reduce inflammation [170]. In a transgenic mouse overexpressing haptoglobin in alveolar macrophages, CFH clearance and decreased lung injury were observed, suggesting that haemoglobin catabolism is linked to iron mobilisation in macrophages [171].

9.5 Lipoxin A4

Lipoxin A4 (LXA4) is derived from arachidonic acid that promotes alveolar epithelial wound repair and the proliferation and differentiation of ATII cells into ATI cells [172]. *In vivo*, LPS-induced ALI model animals resulted in decreased levels of TNF-α and IL-1β, inhibition of neutrophil recruitment to the lung, inhibition of ATII cell apoptosis and epithelial-mesenchymal transition [173, 174].

Resolvin D1 is a specialised pro-resolving mediator that acts by stimulating the lipoxin A4 receptor on immune cells, reducing ROS generation, blocking nuclear factor kappa-light-chain-enhancer of activated B cells (NF-$\kappa\beta$) activation and

accelerating the production of antioxidant proteins. In a mouse model, resolvin D1 treatment reduces leukocyte infiltration and inflammatory cytokine release [175, 176]. In a rat ALI model, resolvin administration attenuated LPS-induced ALI and promoted alveolar fluid clearance by increasing the expression of sodium and Na, K-ATPase channels [177].

10. Conclusion

Management of patients with ARDS has substantially progressed, although this syndrome still remains relatively common, with high associated morbidity, mortality and persisting sequelae on survivors. All these expose the need for effective pharmacological therapies.

Although various treatments have failed when being translated to ARDS patients, other therapies are ongoing and proved efficacy in preclinical and clinical studies. However, there are different factors that should be taken into account for future research, in order to maximise patient treatment response.

Because of ARDS complex pathophysiology, to classify this heterogenic syndrome in identified patient subsets or phenotypes based on clinical, physiologic, radiologic and biologic criteria might result in a more feasible patient response, leading to personalized therapy [178]. In addition, time, dose and pathway administration of treatment are critical. Also, taking into account the complexity of the disease, a unique or combined therapy might encompass various pathways and mechanisms involved in the pathophysiology. Of no less importance, we should be aware that preclinical models reproduce human ARDS only in part, fact that could affect the relevance of the data [179].

Promising therapies for ARDS are underway. Increased knowledge on involved pathways and mechanism of ARDS pathophysiology and the identification of ARDS patient subsets will contribute on the development of effective therapies.

AUTHOR CONTRIBUTIONS

ECD and MCR contributed in the design of the review, searched the literature, studied and interpreted the data and wrote the manuscript. Both authors approved the last version of the manuscript.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

Not applicable.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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